Unnatural Amino Acid Incorporation in Virus-like Particles

Erica Strable,[a] Duane E. Prasuhn Jr.,[a] Andrew K. Udit,[a] Steven Brown,[a] A. James Link,[b] John T. Ngo,[b] Gabriel Lander,[c] Joel Quispe,[c] Clinton S. Potter,[c] Bridget Carragher,[c] David A. Tirrell,[b] M.G. Finn[a]*

[a]Department of Chemistry and The Skaggs Institute of Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037; [b]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125; [c] National Resource for Automated Molecular Microscopy and Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

Supporting Information

1. Expression of Qβ(K16M-CCH)$_{90}$ in M15(pREP4)MA cells.

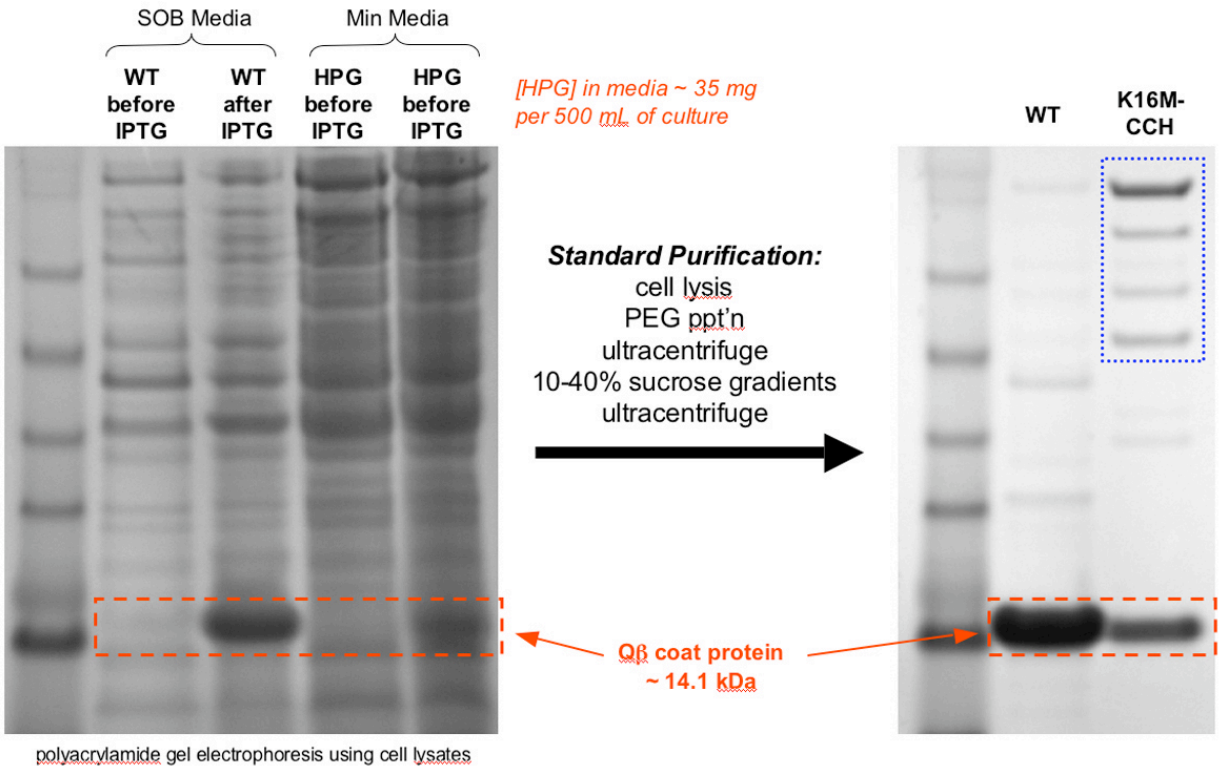

Figure S1. (Left) Gel electrophoresis analyses of cell lysates showing IPTG-induced expression of Qβ coat protein under standard (SOB media) and unnatural (Min media) conditions, the latter in the presence of homopropargylglycine **2**. (Right) Gel electrophoresis analysis of purified Qβ(K16M-CCH)$_{90}$ particles. The bands in the blue box are impurities which are removed by a second sucrose gradient ultracentrifugation.

2. Media for expression of unnatural amino acids in M15(pREP4)MA cells.

M9 Minimal Media	25X M9 Salts	19 Amino Acid Mix
500 mL of doubly deionized H_2O 25 mL 25X M9 salts 25 mL 20% (w/v) glucose 25 mL 19 Amino Acid Mix 2mL 1M MgCl 0.2 mL 1M $CaCl_2$ 4 mL methionine or 40 mg azidohomoalanine	320 g Na_2HPO_4•$7H_20$ 75 g KH_2PO_4 12.5 g NaCl 25 g NH_4Cl doubly deionized H_2O to 1 L pH 7.4	1 L doubly deionized H_2O 2g serine 1 g remaining amino acids minus methionine

3. Characterization of Qβ(K16M-CCH)$_{90}$.

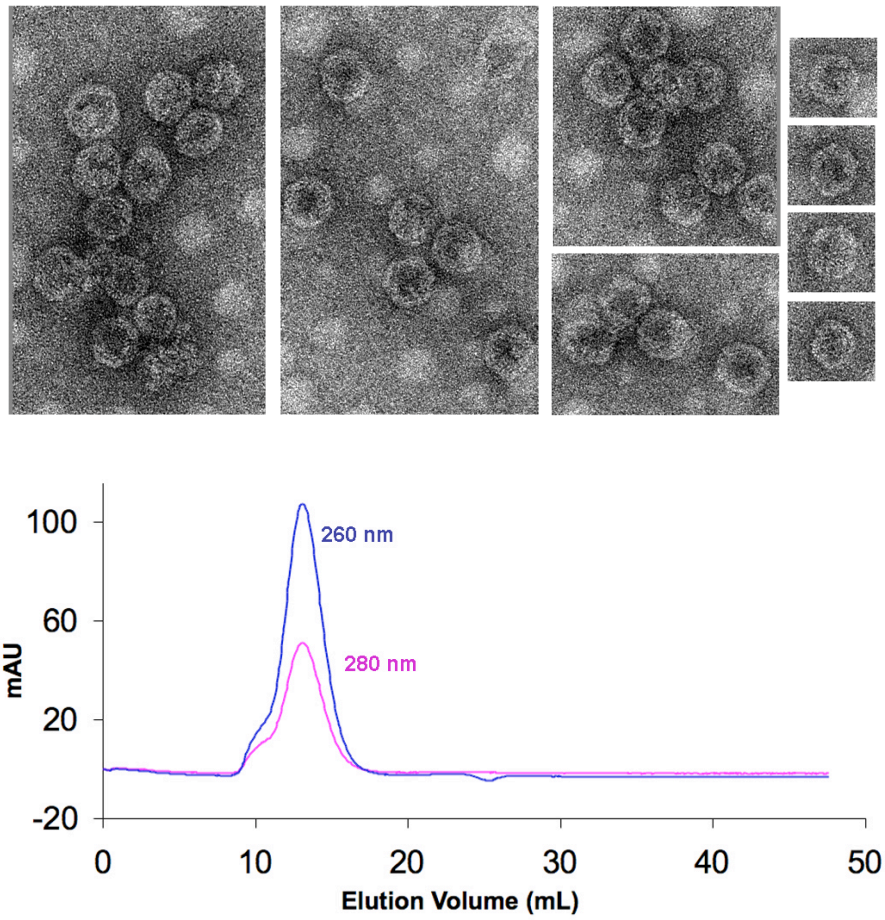

Figure S2. (Top) Negative-stained (1% uranyl acetate) TEM images (45K magnification) of Qβ(K16M-CCH)$_{90}$, showing 28-nm diameter particles. The white circular images are from the background grid and are not due to protein. (Bottom) Size-exclusion chromatography (Superose-6) of Qβ(K16M-CCH)$_{90}$, showing the retention volume and A_{260}/A_{280} ratio characteristic of intact Qβ particles.

*4. Characterization of Qβ(K16M-CCH)$_{90}$ adduct with azide **11**.*

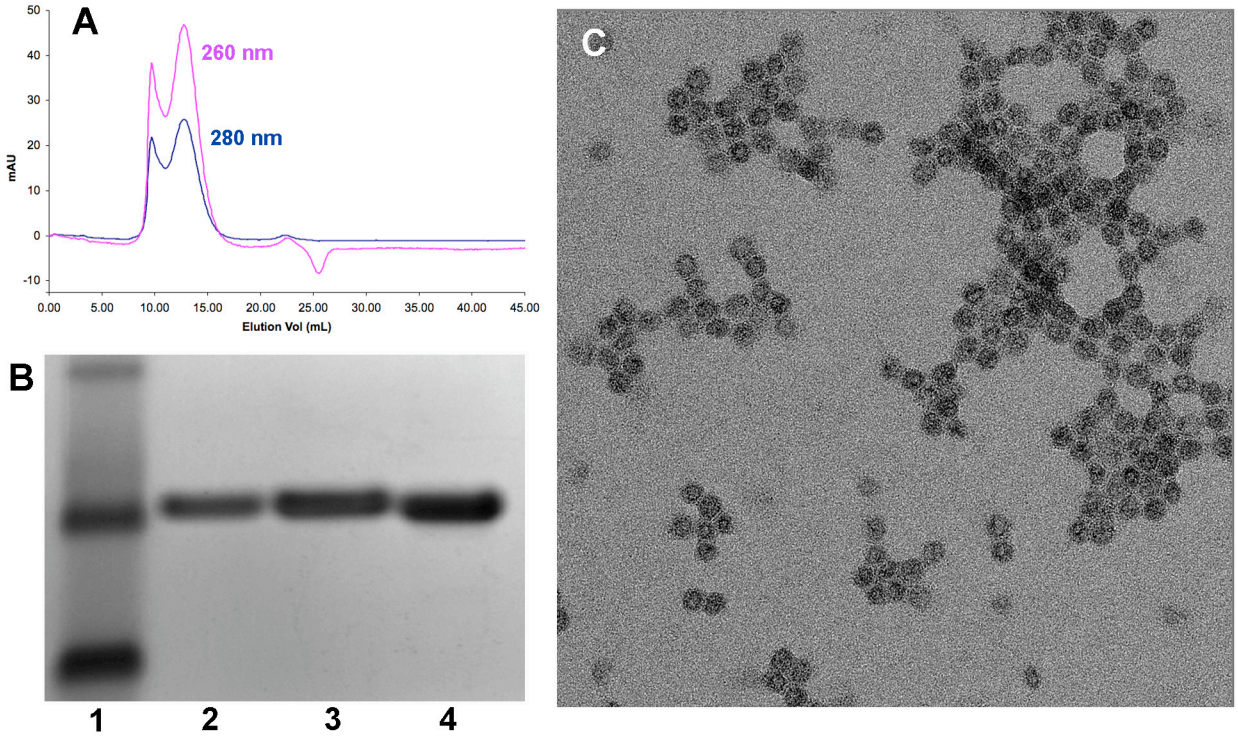

Figure S3. (A) Size-exclusion chromatography (Superose-6) of the purified adduct of Qβ(K16M-CCH)$_{90}$ with azide **11**. This material contained a mixture of two fractions eluted from the 10-40% sucrose gradient, comprising a "tight" band of material at the characteristic density of Qβ VLPs, and a more diffuse band at slightly higher density. The observation of two fractions in the size-exclusion chromatogram suggests some aggregation of particles under both conditions. (B) Denaturing polyacrylamide gel electrophoresis of the purifed adduct in part (A). Lane 1 = ladder; lane 2 = top fraction; lane 3 = bottom fraction; lane 4 = wild-type Qβ, unlabeled. (C) Negative-stained (1% uranyl acetate) TEM image (45K magnification) of the purified adduct in part (A), showing intact particles.

Table S1. Reported yields of proteins incorporating unnatural amino acids. Note that many more examples are known, but these comprise most of those that describe isolated yields of significant amounts of protein. DHFR = dihydrofolate reductase; GFP = green fluorescent protein; Pr A = Z-domain of protein A; SD = superoxide dismutase.

Unnatural Amino Acid Incorporated	Protein	Protein yield (mg/L)	Ref.
3,4-dihydroxy-L-phenylalanine	Myoglobin	1	[1]
p-benzoyl-L-phenylalanine	Myoglobin	2	[2]
p-azido-L-phenylalanine	Myoglobin	2	[3]
^{15}N labeled *p*-methoxyphenylalanine	Myoglobin	1	[4]
p-azido-L-phenylalanine	SD	0.8	[5]
azidohomoalanine	DHFR	35	[6]
2-amino-5-hexynoic acid	DHFR	35	[6]
norleucine	DHFR	20	[6]
homoallylglycine	DHFR	10	[7]
p-iodophenylalanine	DHFR	6-18	[8]
p-cyanophenylalanine	DHFR	6-18	[8]
p-ethynylphenylalanine	DHFR	6-18	[8]
p-azidophenylalainine	DHFR	6-18	[8]
2-pyrindinylalanine	DHFR	6-18	[8]
3-pyrindinylalanine	DHFR	6-18	[8]
4-pyrindinylalanine	DHFR	6-18	[8]
p-acetyl-L-phenylalanine	Pr A	3.6	[9]
(2S,3S)-2-aminomethyl pentanoic acid	DHFR	12-22	[10]
trifluoroisoleucine	DHFR	20	[11]
p-nitrophenylalanine	Pr A	2	[12]
homoallylglycine	DHFR	70	[13, 14]
2-amino-5-hexynoic acid	DHFR	8	[13]
L-3-(2-napthyl)alanine	DHFR	2.2	[15]
p-amino-L-phenylalanine	GFP	10-22	[16]
p-methoxy-L-phenylalanine	GFP	10-22	[16]
p-iodo-L-phenylalanine	GFP	10-22	[16]
p-bromo-L-phenylalanine	GFP	10-22	[16]
L-3-(2-napthyl)alanine	GFP	10-22	[16]
p-acetyl-phenylalanine	Pr A	3.6-9.2	[17]
o-methyltyrosine	SD	0.6	[18]
α-aminocaprylic acid	SD	0.6	[18]
o-nitrobenzyl cysteine	SD	0.6	[18]
p-iodo-L-phenylalanine	Pr A	3.8-4.6	[19]
p-iodo-L-phenylalanine	T4 Lysozyme	5.7	[19]
N-acetylgalactosamine-α-*o*-threonine	Myoglobin	2.4	[20]

1. Alfonta, L., Zhang, Z., Uryu, S., Loo, J.A., and Schultz Peter, G. (2003). Site-Specific Incorporation of a Redox-Active Amino Acid into Proteins. *J. Am. Chem. Soc. 125*, 14662-14663.
2. Chin, J.W., Martin, A.B., King, D.S., Wang, L., and Schultz, P.G. (2002). Addition of a photocrosslinking amino acid to the genetic code of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA 99*, 11020-11024.
3. Chin, J.W., Santoro Stephen, W., Martin Andrew, B., King David, S., Wang, L., and Schultz Peter, G. (2002). Addition of p-Azido-L-phenylalanine to the Genetic Code of *Escherichia coli*. *J. Am. Chem. Soc. 124*, 9026-9027.
4. Deiters, A., Geierstanger, B.H., and Schultz, P.G. (2005). Site-specific in vivo labeling of proteins for NMR studies. *ChemBioChem 6*, 55-58.
5. Deiters, A., Cropp, T.A., Summerer, D., Mukherji, M., and Schultz, P.G. (2004). Site-specific PEGylation of proteins containing unnatural amino acids. *Bioorg. Med. Chem. Lett*. *14*, 5743-5745.
6. Kiick, K.L., Saxon, E., Tirrell, D.A., and Bertozzi, C.R. (2002). Incorporation of azides into recombinant proteins for chemoselective modification by the Staudinger ligation. *Proc. Natl. Acad. Sci. USA 99*, 19-24.
7. Kiick, K., L., and Tirrell David, A. (2000). Protein Engineering by In Vivo Incorporation of Non-Natural Amino Acids: Control of Incorporation of Methioonine Analogues by Methionyl tRNA Synthetase. *Tetrahedron 56*, 9487-9493.
8. Kirshenbaum, K., Carrico Isaac, S., and Tirrell David, A. (2002). Biosynthesis of proteins incorporating a versatile set of phenylalanine analogues. *ChemBioChem 3*, 235-237. .
9. Liu, H., Wang, L., Brock, A., Wong, C.-H., and Schultz, P.G. (2003). A method for the generation of glycoprotein mimetics. *J. Am. Chem. Soc. 125*, 1702-1703.
10. Mock, M.L., Michon, T., van Hest, J.C.M., and Tirrell, D.A. (2006). Stereoselective incorporation of an unsaturated isoleucine analogue into a protein expressed in *E. coli. ChemBioChem 7*, 83-87.
11. Wang, P., Tang, Y., and Tirrell, D.A. (2003). Incorporation of Trifluoroisoleucine into Proteins in Vivo. *J. Am. Chem. Soc. 125*, 6900-6906.
12. Tsao, M.-L., Summerer, D., Ryu, Y., and Schultz, P.G. (2006). The Genetic Incorporation of a Distance Probe into Proteins in *Escherichia coli*. *J. Am. Chem. Soc. 128*, 4572-4573.
13. Van Hest, J.C.M., Kiick, K.L., and Tirrell, D.A. (2000). Efficient incorporation of unsaturated methionine analogues into proteins *in vivo. J. Am. Chem. Soc. 122*, 1282-1288.
14. van Hest, J.C.M., and Tirrell, D.A. (1998). Efficient introduction of alkene functionality into proteins *in vivo. FEBS Lett. 428*, 68-70.
15. Wang, L., Brock, A., and Schultz, P.G. (2002). Adding L-3-(2-Naphthyl)alanine to the genetic code of *Escherichia coli. J. Am. Chem. Soc. 124*, 1836-1837.
16. Wang, L., Xie, J., Deniz, A.A., and Schultz, P.G. (2003). Unnatural Amino Acid Mutagenesis of Green Fluorescent Protein. *J. Org. Chem*. *68*, 174-176.
17. Wang, L., Zhang, Z., Brock, A., and Schultz, P.G. (2003). Addition of the keto functional group to the genetic code of *Echerichia coli*. *Proc. Natl. Acad. Sci. USA 100*, 56-61.
18. Wu, N., Deiters, A., Cropp, T.A., King, D., and Schultz, P.G. (2004). A Genetically Encoded Photocaged Amino Acid. *J. Am. Chem. Soc. 126*, 14306-14307.
19. Xie, J., Wang, L., Wu, N., Brock, A., Spraggon, G., and Schultz, P.G. (2004). The site-specific incorporation of p-iodo-L-phenylalanine into proteins for structure determination. *Nat. Biotech*. *22*, 1297-1301.
20. Xu, R., Hanson, S.H., Zhang, Z., Yang, Y.-Y., Schultz Peter, G., and Wong, C.-H. (2004). Site-Specific Incorporation of the Mucin-Type-N-Acetylgalactosamin-α-*o*-threonine into Protein in *Escherichia coli*. *J. Am. Chem. Soc. 126*, 15654-15655.